Formulation containing anti-inflammatory androstane derivative

This application is a Continuation-in-part of a US 35 USC 371 patent application, Serial No. 09/958050 filed on 2 October 2001, for which an International Patent Application No. PCT.GB01.03495 filed 3 August 2001, which claims priority to United Kingdom Patent Application No. GB 0019172.6 filed 5 August 2000.

The present invention relates to pharmaceutical formulations containing an anti-inflammatory and anti-allergic compound of the androstane series and to processes for their preparation. The present invention also relates to therapeutic uses thereof, particularly for the treatment of inflammatory and allergic conditions.

Glucocorticoids which have anti-inflammatory properties are known and are widely used for the treatment of inflammatory disorders or diseases such as asthma and rhinitis. For example, US Patent 4335121 discloses 6α, 9α-Difluoro-17α-(1-oxopropoxy)-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester (known by the generic name of fluticasone propionate) and derivatives thereof. The use of glucocorticoids generally, and especially in children, has been limited in some quarters by concerns over potential side effects. The side effects that are feared with glucocorticoids include suppression of the Hypothalamic-Pituitary-Adrenal (HPA) axis, effects on bone growth in children and on bone density in the elderly, ocular complications (cataract formation and glaucoma) and skin atrophy. Certain glucocorticoid compounds also have complex paths of metabolism wherein the production of active metabolites may make the pharmacodynamics and pharmacokinetics of such compounds difficult to understand. Whilst the modern glucocorticoids are very much safer than those originally introduced, it remains an object of research to produce new molecules and formulations of old and new molecules which have excellent anti-inflammatory properties, with predictable pharmacokinetic and pharmacodynamic properties, with an attractive side effect profile, and with a convenient treatment regime.

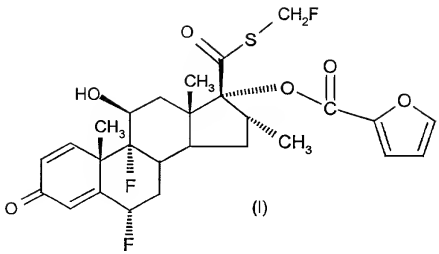
We have now identified a novel glucocorticoid compound and formulation thereof which substantially meets these objectives, in particular one suitable for intranasal administration.

Many millions of individuals suffer from seasonal and perennial allergic rhinitis worldwide. Symptoms of seasonal and perennial allergic rhinitis include nasal itch, congestion, runny nose, sneezing and watery eyes. Seasonal allergic rhinitis is commonly known as 'hay fever'. It is caused by allergens which are present in the air at specific times of the year, for example tree pollen during Spring and Summer. Perennial allergic rhinitis is caused by allergens which are present in the environment during the entire year, for example dust mites, mold, mildew and pet dander.

To formulate an effective pharmaceutical nasal composition, the medicament must be delivered readily to all portions of the nasal cavities (the target tissues) where it performs its pharmacological function. Additionally, the medicament should remain in contact with the target tissues for relatively long periods of time. The longer the medicament remains in contact with the target tissues, the medicament must be capable of resisting those forces in the nasal passages that function to remove particles from the nose. Such forces, referred to as 'mucociliary clearance', are recognised as being extremely effective in removing particles from the nose in a rapid manner, for example, within 10-30 minutes from the time the particles enter the nose.

Other desired characteristics of a nasal composition are that it must not contain ingredients which cause the user discomfort, that it has satisfactory stability and shelf-life properties, and that it does not include constituents that are considered to be detrimental to the environment, for example ozone depletors. In the case of administration of glucocorticoids, the potential for any undesirable side effects should preferably be minimised.

Thus, according to one aspect of the invention, there is provided a pharmaceutical formulation comprising an aqueous suspension of particulate compound of formula (I)



(I)

or a solvate thereof.

Preferably, the formulation will contain one or more suspending agents.

Preferably, the formulation will contain one or more preservatives.

Preferably, the formulation will contain one or more wetting agents.

Preferably, the formulation will contain one or more isotonicity adjusting agents.

According to one particular aspect of the present invention we provide a pharmaceutical formulation which comprises:

(i) an aqueous suspension of particulate compound of formula (I) or a solvate thereof;

(ii) one or more suspending agents;

(iii) one or more preservatives;

(iv) one or more wetting agents; and

(v) one or more isotonicity adjusting agents.

The formulations of the present invention may be stabilised by appropriate selection of pH using hydrochloric acid. Typically, the pH will be adjusted to between 4.5 and 7.5, preferably between 5.0 and 7.0, especially around 6.5.

Examples of pharmaceutically acceptable materials which can be used to adjust the pH of the formulation include hydrochloric acid and sodium hydroxide. Preferably, the pH of the formulation will be adjusted using hydrochloric acid.

The aqueous component is preferably a high grade quality of water, most preferably purified water.

The active compound of formula (I) or solvate thereof will suitably have a mass mean diameter (MMD) of less than 20μm, preferably between 0.5-10μm, especially around 3-5μm, eg. 2μm. Particle size reduction, if necessary, may be achieved eg. by micronisation. Preferably, the particles will be crystalline, prepared for example by a process which comprises mixing in a continuous flow cell in the presence of ultrasonic radiation a flowing solution of compound of formula (I) or solvate thereof as medicament in a liquid solvent with a flowing liquid antisolvent for said medicament (as described in International Patent Application PCT/GB99/04368).

A pharmaceutically effective amount of particulate compound of formula (I) or solvate thereof is present within the formulation, in an amount which is preferably between 0.005% and 1% (w/w), preferably between 0.01% and 0.5% (w/w), especially 0.05-0.1% (w/w) based on the total weight of the formulation. Typically, 50μl of suspension will contain 50μg of compound of formula (I) or solvate thereof.

Examples of suspending agents include carboxymethylcellulose, veegum, tragacanth, bentonite, methylcellulose and polyethylene glycols. Preferably, the suspending agent will be microcrystalline cellulose and carboxy methylcellulose sodium, most preferably used as the branded product Avicel RC591 (which typically contains 87-91% microcrystalline cellulose and 9 -13% carboxy methylcellulose sodium). Particulate microcrystalline cellulose will preferably have a particle size in the range 1 to 100μm. We believe that Avicel RC591 acts as a suspending agent by imparting thixotropic properties to the formulation, wherein the formulation may become a stable suspension upon being stirred, shaken or otherwise disturbed.

Preferably, the thixotropic nature of the suspending agent will ensure that the formulation assumes a gel like appearance at rest, wherein the particulate medicament is dispersed and suspended substantially uniformly, characterised by a high viscosity value. Once the composition is subjected to shear forces, such as those caused by agitation prior to spraying, the viscosity of the formulation will preferably decrease to such a level to enable it to flow readily through the spray device and exit as a spray of fine particles in a mist. These particles will then be capable of infiltrating the mucosal surfaces of the anterior regions of the nose (frontal nasal cavities), the frontal sinus, the maxillary sinuses and the turbinates which overlie the conchas of the nasal cavities. Once deposited, the viscosity of the formulation will preferably increase to a sufficient level to assume its gel-like form and resist being cleared from the nasal passages by the inherent mucocillary forces that are present in the nasal cavities.

When the formulation of the present invention comprises a suspending agent, it will be desirably added in a suitable amount to achieve this function, preferably the suspending agent will be present within the formulation in an amount of between 0.1 and 5% (w/w), especially 1.5% (w/w), based on the total weight of the formulation.

For stability purposes, the formulation of the present invention should be protected from microbial contamination and growth. Examples of pharmaceutically acceptable anti-microbial agents that can be used in the formulation include quaternary ammonium compounds (eg. benzalkonium chloride, benzethonium chloride, cetrimide and cetylpyridinium chloride), mercurial agents (eg. phenylmercuric nitrate, phenylmercuric acetate and thimerosal), alcoholic agents (eg. chlorobutanol, phenylethyl alcohol and benzyl alcohol), antibacterial esters (eg. esters of para-hydroxybenzoic acid), chelating agents such as disodium edetate (EDTA) and other anti-microbial agents such as chlorhexidine, chlorocresol, sorbic acid and its salts and polymyxin.

Preferably, the preservative will comprise disodium edetate, which will preferably be present within the formulation in an amount of between 0.001 and 1% (w/w), especially around 0.015% (w/w), based on the total weight of the formulation.

Preferably the preservative will comprise potassium sorbate which will preferably be present within the formultaion in an amount of between 0.01 and 1% (w/w), especially around 0.015% (w/w) based on the total weight of the formulation.

Preferably, the preservative will comprise benzalkonium chloride (BKC), which will preferably be present within the formulation in an amount of between 0.001 and 1% (w/w), especially around 0.015% (w/w), based on the total weight of the formulation.

More preferably, the preservative comprises disodium edetate and benzalkonium chloride or disodium edetate and potassium sorbate, particularly disodium edetate and benzalkonium chloride.

Formulations, eg nasal formulations which contain a suspended medicament (such as a compound of formula (I) or a solvate thereof) will preferably contain a pharmaceutically acceptable wetting agent which functions to wet the particles of medicament to facilitate dispersion thereof in the aqueous phase of the composition. Preferably, the amount of wetting agent used will not cause foaming of the dispersion during mixing.

It will be appreciated that any agent which is effective in wetting the particles and which is pharmaceutically acceptable can be used. Examples of wetting agents that can be used are fatty alcohols, esters and ethers. Preferably, the wetting agent will be a hydrophilic, non-ionic surfactant, most preferably polyoxyethylene (20) sorbitan monooleate (supplied as the branded product Polysorbate 80).

Wherein the formulation of the present invention comprises a wetting agent, it will be desirably added in a sufficient quantity to achieve this function, preferably the wetting agent will be present within the formulation in an amount of between 0.001 and 0.05% (w/w), especially 0.025% (w/w), based on the total weight of the formulation.

The presence of an isotonicity adjusting agent is to achieve isotonicity with body fluids eg fluids of the nasal cavity, resulting in reduced levels of irritancy associated

with many nasal formulations. Examples of suitable isotonicity adjusting agents are sodium chloride, dextrose and calcium chloride. Preferably, the isotonicity adjusting agent will be dextrose, most preferably used as dextrose anhydrous.

When the formulation of the present invention comprises an isotonicity adjusting agent it will be desirably added in a sufficient quantity to achieve this function, preferably the isotonicity adjusting agent will be present within the formulation in an amount of between 0.1 and 10% (w/w), especially 5.0% w/w, based on the total weight of the formulation.

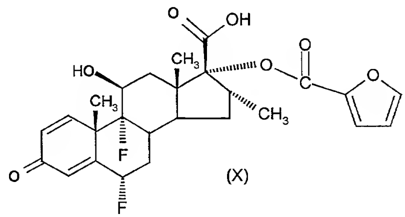
The compound of formula (I) or a solvate thereof and formulations thereof have potentially beneficial anti-inflammatory or anti-allergic effects, particularly upon topical administration to the nose, demonstrated by, for example, its ability to bind to the glucocorticoid receptor and to illicit a response via that receptor, with long acting effect. Hence, formulations according to the invention are useful in the treatment of inflammatory and/or allergic disorders of the nose, especially in once-per-day therapy.

Formulations according to the invention may be prepared by combining the ingredients in water. If necessary the pH may be adjusted as a final step. Formulations so prepared may then be filled into the receptacle.

Aqueous formulations of the invention may also be employed for rectal, aural, otic, oral, topical or parenteral administration or administration by inhalation for the treatment of other local inflammatory conditions (eg dermatitis, asthma, chronic obstructive pulmonary disease (COPD) and the like). For example formulations of the invention may be administered to the lung by nebulisation. Such formulations may employ excipients (eg preservatives, buffers and the like) appropriate for the route of administration.

The particularly desirable biological properties of the compound of formula (I) are now explained below:

Compound or formula (I) undergoes highly efficient hepatic metabolism to yield the 17-β carboxylic acid (X) as the sole major metabolite in rat and human *in vitro* systems. This metabolite has been synthesised and demonstrated to be >1000 fold less active than the parent compound in *in vitro* functional glucocorticoid assays.



(X)

This efficient hepatic metabolism is reflected by *in vivo* data in the rat, which have demonstrated plasma clearance at a rate approaching hepatic blood flow and an oral bioavailability of <1%, consistent with extensive first-pass metabolism.

In vitro metabolism studies in human hepatocytes have demonstrated that compound (I) is metabolised in an identical manner to fluticasone propionate but that conversion of (I) to the inactive acid metabolite occurs approximately 5-fold more rapidly than with fluticasone propionate. This very efficient hepatic inactivation would be expected to minimise systemic exposure in man leading to an improved safety profile.

Inhaled glucocorticoids are also absorbed through the lung and this route of absorption makes a significant contribution to systemic exposure. Reduced lung absorption could therefore provide an improved safety profile. Studies with compound of formula (I) have shown significantly lower exposure to compound of formula (I) than with fluticasone propionate after dry powder delivery to the lungs of anaesthetised pigs.

Examples of disease states in which the compound of formula (I) has utility include inflammatory and/or allergic conditions of the nasal passages such as rhinitis eg seasonal and perennial rhinitis as well as other local inflammatory conditions such as asthma, COPD and dermatitis.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established conditions.

Preferable means for applying the formulation of the present invention to the nasal passages is by use of a pre-compression pump. Most preferably, the pre-compression pump will be a VP7 model manufactured by Valois SA. Such a pump is beneficial as it will ensure that the formulation is not released until a sufficient force has been applied, otherwise smaller doses may be applied. Another advantage of the pre-compression pump is that atomisation of the spray is ensured as it will not release the formulation until the threshold pressure for effectively atomising the spray has been achieved. Typically, the VP7 model may be used with a botte capable of holding 10-50ml of a formulation. Each spray will typically deliver 50-100μl of such a formulation, therefore, the VP7 model is capable of providing at least 100 metered doses.

A suitable dosing regime for the formulation of the present invention when administered to the nose would be for the patient to inhale deeply subsequent to the nasal cavity being cleared. During inhalation the formulation would be applied to one nostril while the other is manually compressed. This procedure would then be repeated for the other nostril.

Typically, one or two inhalations per nostril would be administered by the above procedure up to three times each day, preferably once or twice daily, especially once daily.

It will be appreciated that the above dosing regime should be adjusted according to the patient's age, body weight and/or symptom severity.

As mentioned above, formulations comprising a compound of formula (I) or solvate thereof are useful in human or veterinary medicine, in particular as an anti-inflammatory and anti-allergic agent.

There is thus provided as a further aspect of the invention a formulation comprising the compound of formula (I) or solvate thereof for use in human or veterinary medicine, particularly in the treatment of patients with inflammatory and/or allergic conditions.

According to another aspect of the invention, there is provided the use of a formulation comprising the compound of formula (I) or solvate thereof for the manufacture of a medicament for the treatment of patients with inflammatory and/or allergic conditions.

In a further or alternative aspect, there is provided a method for the treatment of a human or animal subject with an inflammatory and/or allergic condition, which method comprises administering to said human or animal subject an effective amount of a formulation comprising the compound of formula (I) or solvate thereof.

The compound of formula (I) is long-acting, therefore preferably the compound will be delivered once-per-day and the dose will be selected so that the compound has a therapeutic effect in the treatment of respiratory disorders (eg rhinitis) over 24 hours or more.

The pharmaceutical compositions according to the invention may also be used in combination with another therapeutically active agent, for example, an anti-histamine or an anti-allergic. The invention thus provides, in a further aspect, a combination comprising the compound of formiula (I) or solvate thereof together with another therapeutically active agent, for example, an anti-histamine or an anti-allergic.

Examples of anti-histamines include methapyrilene or loratadine.

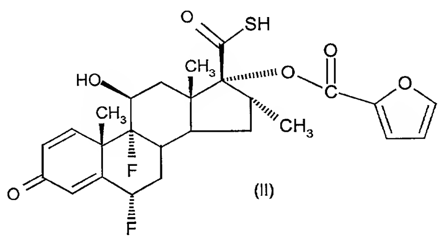
Other suitable combinations include, for example, other anti-inflammatory agents eg NSAIDs (eg sodium cromoglycate, nedocromil sodium, PDE4 inhibitors, leukotriene antagonists, iNOS inhibitors, tryptase and elastase inhibitors, beta-2 integrin antagonists and adenosine 2a agonists)) or antiinfective agents (eg antibiotics, antivirals).

Also of particular interest is use of the compound of formula (I) or a solvate thereof in combination with a phosphodiesterase 4 (PDE4) inhibitor eg cilomilast or a salt thereof.

Further, there is provided a process for the preparation of such pharmaceutical compositions which comprises mixing the ingredients.

The individual compounds of such combinations may be administered either sequentially in separate pharmaceutical compositions as well as simultaneously in combined pharmaceutical formulations. Preferably additional therapeutically active ingredients are suspended in the formulation together with the compound of formula (I) Appropriate doses of known therapeutic agents will be readily appreciated by those skilled in the art.

A process for preparing a compound of formula (I) comprises alkylation of a thioacid of formula (II)



(II)

or a salt thereof.

In this process the compound of formula (II) may be reacted with a compound of formula FCH_2L wherein L represents a leaving group (eg a halogen atom, a mesyl or tosyl group or the like) for example, an appropriate fluoromethyl halide under standard conditions. Preferably, the fluoromethyl halide reagent is bromofluoromethane. Preferably the compound of formula (II) is employed as a salt, particularly the salt with diisopropylethylamine.

In a preferred process for preparing the compound of formula (I), the compound of formula (II) or a salt thereof is treated with bromofluoromethane optionally in the presence of a phase transfer catalyst. A preferred solvent is methylacetate, or more preferably ethylacetate, optionally in the presence of water. The presence of water improves solubility of both starting material and product and the use of a phase transfer catalyst results in an increased rate of reaction. Examples of phase transfer catalysts that may be employed include (but are not restricted to) tetrabutylammonium bromide, tetrabutylammonium chloride, benzyltributylammonium bromide, benzyltributylammonium chloride, benzyltriethylammonium bromide, methyltributylammonium chloride and methyltrioctylammonium chloride. THF has also successfully been employed as solvent for the reaction wherein the presence of a phase transfer catalyst again provides a significantly faster reaction rate. Preferably the product present in an organic phase is washed firstly with aqueous acid eg dilute HCl in order to remove amine compounds such as triethylamine and diisopropylethylamine and then with aqueous base eg sodium bicarbonate in order to remove any unreacted precursor compound of formula (II).

Compound of formula (I) in unsolvated form may be prepared by a process comprising:

(a) Crystallising the compound of formula (I) in the presence of a non-solvating solvent such as ethanol, methanol, water, ethyl acetate, toluene, methylisobutylketone or mixtures thereof; or

(b) Desolvating a compound of formula (I) in solvated form (eg in the form of a solvate with acetone, isopropanol, methylethylketone, DMF or tetrahydrofuran) eg by heating.

In step (b) the desolvation will generally be performed at a temperature exceeding 50 °C preferably at a temperature exceeding 100 °C. Generally heating will be performed under vacuum.

Compound of formula (I) in unsolvated form has been found to exist in 3 crystalline polymorphic forms, Forms 1, 2 and 3, although Form 3 may be an unstable variant of

Form 2. The Forms are characterised by their X-ray diffraction (XRPD) patterns Broadly speaking the Forms are characterised in their XRPD profiles as follows:

Form 1: Peak at around 18.9 degrees 2Theta

Form 2: Peaks at around 18.4 amd 21.5 degrees 2Theta

Form 3: Peaks at around 18.6 and 19.2 degrees 2Theta.

Forms 1 appears likely to be the thermodynamically most stable form since Forms 2 and 3 are converted into Form 1 on heating.

A process for preparing a compound of formula (I) as unsolvated Form 1 polymorph comprises dissolving compound of formula (I) in methylisobutylketone, ethyl acetate or methyl acetate and producing compound of formula (I) as unsolvated Form 1 by addition of a non-solvating anti-solvent such as iso-octane or toluene.

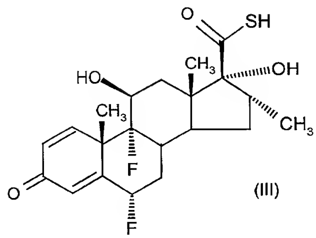
According to a first preferred embodiment of this process the compound of formula (I) may be dissolved in ethyl acetate and compound of formula (I) as unsolvated Form 1 polymorph may be obtained by addition of toluene as anti-solvent. In order to improve the yield, preferably the ethyl acetate solution is hot and once the toluene has been added the mixture is distilled to reduce the content of ethyl acetate.

According to a second preferred embodiment of this process the compound of formula (I) may be dissolved in methylisobutylketone and compound of formula (I) as unsolvated Form 1 polymorph may be obtained by addition of isooctane as anti-solvent

Compound of formula (I) in solvated form may be prepared by crystallising the compound of formula (I) from a solvating solvent such as acetone or tetrahydrofuran (THF).

Preferably in processes for preparing formulations of the invention, the compound of formula (I) will be employed in unsolvated form, typically unsolvated Form 1.

Compounds of formula (II) may be prepared from the corresponding 17α-hydroxyl derivative of formula (III):



(III)

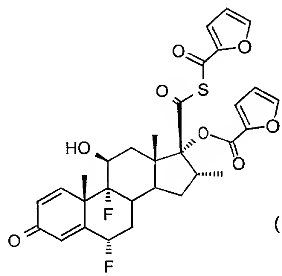
using for example, the methodology described by G. H. Phillipps et al., (1994) Journal of Medicinal Chemistry, **37**, 3717-3729. For example the step typically comprises the addition of a reagent suitable for performing the esterification eg an activated derivative of 2-furoic acid such as an activated ester or preferably a 2-furoyl halide eg 2-furoyl chloride (employed in at least 2 times molar quantity relative to the compound of formula (III)) in the presence of an organic base eg triethylamine. The second mole of 2-furoyl chloride reacts with the thioacid moiety in the compound of formula (III) and needs to be removed eg by reaction with an amine such as diethylamine.

This method suffers disadvantages, however, in that the resultant compound of formula (II) is not readily purified of contamination with the by-product 2-furoyldiethylamide. We have therefore invented several improved processes for performing this conversion.

In a first such improved process we have discovered that by using a more polar amine such as diethanolamine, a more water soluble by-product is obtained (in this case 2-furoyldiethanolamide) which permits compound of formula (II) or a salt thereof to be produced in high purity since the by-product can efficiently be removed by water washing.

Thus we provide a process for preparing a compound of formula (II) which comprises:

(a) reacting a compound of formula (III) with an activated derivative of 2-furoic acid as in an amount of at least 2 moles of the activated derivative per mole of compound of formula (III) to yield a compound of formula (IIA)



(IIA)

; and

(b) removal of the sulphur-linked 2-furoyl moiety from compound of formula (IIA) by reaction of the product of step (a) with an organic primary or secondary amine base capable of forming a water soluble 2-furoyl amide.

In two particularly convenient embodiments of this process we also provide methods for the efficient purification of the end product which comprise either

(c1)when the product of step (b) is dissolved in a substantially water immiscible organic solvent, purifying the compound of formula (II) by washing out the amide by-product from step (b) with an aqueous wash, or

(c2) when the product of step (b) is dissolved in a water miscible solvent, purifying the compound of formula (II) by treating the product of step (b) with an aqueous medium so as to precipitate out pure compound of formula (II) or a salt thereof.

In step (a) preferably the activated derivative of 2-furoic acid may be an activated ester of 2-furoic acid, but is more preferably a 2-furoyl halide, especially 2-furoyl chloride. A suitable solvent for this reaction is ethylacetate or methylacetate (preferably methylacetate) (when step (c1) may be followed) or acetone (when step (c2) may be followed). Normally an organic base eg triethylamine will be present. In step (b) preferably the organic base is diethanolamine. The base may suitably be

dissolved in a solvent eg methanol. Generally steps (a) and (b) will be performed at reduced temperature eg between 0 and 5 °C. In step (c1) the aqueous wash may be water, however the use of brine results in higher yields and is therefore preferred. In step (c2) the aqueous medium is for example a dilute aqueous acid such as dilute HCl.

We also provide an alternative process for preparing a compound of formula (II) which comprises:

(a) reacting a compound of formula (III) with an activated derivative of 2-furoic acid in an amount of at least 2 moles of activated derivative per mole of compound of formula (III) to yield a compound of formula (IIA); and

(b) removal of the sulphur-linked 2-furoyl moiety from compound of formula (IIA) by reaction of the product of step (a) with a further mole of compound of formula (III) to give two moles of compound of formula (II).

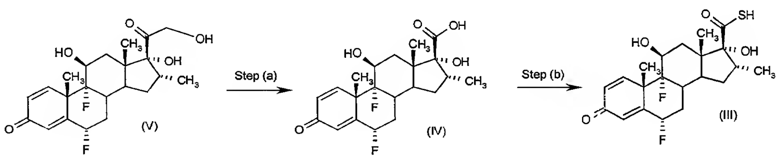
In step (a) preferably the activated derivative of 2-furoic acid may be an activated ester of 2-furoic acid, but is more preferably a 2-furoyl halide, especially 2-furoyl chloride. A suitable solvent for his step is acetone. Normally an organic base eg triethylamine will be present. In step (b) a suitable solvent is DMF or dimethylacetamide. Normally an organic base eg triethylamine will be present. Generally steps (a) and (b) will be performed at reduced temperature eg between 0 and 5 °C. The product may be isolated by treatment with acid and washing with water.

This aforementioned process is very efficient in that it does not produce any furoylamide by-product (thus affording *inter alia* environmental advantages) since the excess mole of furoyl moiety is taken up by reaction with a further mole of compound of formula (II) to form an additional mole of compound of formula (II).

Further general conditions for the conversion of compound of formula (III) to compound of formula (II) in the two processes just described will be well known to persons skilled in the art.

According to a preferred set of conditions, however, we have found that the compound of formula (II) may advantageously be isolated in the form of a solid crystalline salt. The preferred salt is a salt formed with a base such as triethylamine, 2,4,6-trimethylpyridine, diisopropylethylamine or N-ethylpiperidine. Such salt forms of compound of formula (II) are more stable, more readily filtered and dried and can be isolated in higher purity than the free thioacid. The most preferred salt is the salt formed with diisopropylethylamine. The triethylamine salt is also of interest.

Compounds of formula (III) may be prepared in accordance with procedures described in GB 2088877B. Compounds of formula (III) may also be prepared by a process comprising the following steps:



Step (a) comprises oxidation of a solution containing the compound of formula (V). Preferably, step (a) will be performed in the presence of a solvent comprising methanol, water, tetrahydrofuran, dioxan or diethylene glygol dimethylether. So as to enhance yield and throughput, preferred solvents are methanol, water or tetrahydrofuran, and more preferably are water or tetrahydrofuran, especially water and tetrahydrofuran as solvent. Dioxan and diethylene glygol dimethylether are also preferred solvents which may optionally (and preferably) be employed together with water. Preferably, the solvent will be present in an amount of between 3 and 10vol relative to the amount of the starting material (1wt.), more preferably between 4 and 6 vol., especially 5 vol. Preferably the oxidising agent is present in an amount of 1-9 molar equivalents relative to the amount of the starting material. For example, when a 50% w/w aqueous solution of periodic acid is employed, the oxidising agent may be present in an amount of between 1.1 and 10wt. relative to the amount of the starting material (1wt.), more preferably between 1.1 and 3wt., especially 1.3wt. Preferably, the oxidation step will comprise the use of a chemical oxidising agent. More

preferably, the oxidising agent will be periodic acid or iodic acid or a salt thereof. Most preferably, the oxidising agent will be periodic acid or sodium periodate, especially periodic acid. Alternatively (or in addition), it will also be appreciated that the oxidation step may comprise any suitable oxidation reaction, eg one which utilises air and/or oxygen. When the oxidation reaction utilises air and/or oxygen, the solvent used in said reaction will preferably be methanol. Preferably, step (a) will involve incubating the reagents at room temperature or a little warmer, say around 25°C eg for 2 hours. The compound of formula (IV) may be isolated by recrystallisation from the reaction mixture by addition of an anti-solvent. A suitable anti-solvent for compound of formula (IV) is water. Surprisingly we have discovered that it is highly desirable to control the conditions under which the compound of formula (IV) is precipitated by addition of anti-solvent eg water. When the recrystallisation is performed using chilled water (eg water/ice mixture at a temperature of 0-5 °C) although better anti-solvent properties may be expected we have found that the crystalline product produced is very voluminous, resembles a soft gel and is very difficult to filter. Without being limited by theory we believe that this low density product contains a large amount of solvated solvent within the crystal lattice. By contrast when conditions of around 10 °C or higher are used (eg around ambient temperature) a granular product of a sand like consistency which is very easily filtered is produced. Under these conditions, crystallisation typically commences after around 1 hour and is typically completed within a few hours (eg 2 hours). Without being limited by theory we believe that this granular product contains little or no solvated solvent within the crystal lattice.

Step (b) will typically comprise the addition of a reagent suitable for converting a carboxylic acid to a carbothioic acid eg using hydrogen sulphide gas together with a suitable coupling agent eg carbonyldiimidazole (CDI) in the presence of a suitable solvent eg dimethylformamide.

The advantages of the formulation of the compound of formula (I) according to the invention may include the fact that the formulations demonstrate excellent anti-inflammatory properties, with predictable pharmacokinetic and pharmacodynamic behaviour, with an attractive side-effect profile, rapid onset of action, long duration of

action, and are compatible with a convenient regime of treatment in human patients, in particular being amendable to once-per day dosing. Further advantages may include the fact that the formulation has desirable physical and chemical properties which allow for ready manufacture and storage.

The following non-limiting Examples illustrate the invention:

EXAMPLES

General

^{1}H-nmr spectra were recorded at 400 MHz and the chemical shifts are expressed in ppm relative to tetramethylsilane. The following abbreviations are used to describe the multiplicities of the signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dt (doublet of triplets) and b (broad). Biotage refers to prepacked silica gel cartridges containing KP-Sil run on flash 12i chromatography module. LCMS was conducted on a Supelcosil LCABZ+PLUS column (3.3 cm x 4.6 mm ID) eluting with 0.1% HCO_2H and 0.01 M ammonium acetate in water (solvent A), and 0.05% HCO_2H 5% water in acetonitrile (solvent B), using the following elution gradient 0-0.7 min 0%B, 0.7-4.2 min 100%B, 4.2-5.3 min 0%B, 5.3-5.5 min 0%B at a flow rate of 3 ml/min. The mass spectra were recorded on a Fisons VG Platform spectrometer using electrospray positive and negative mode (ES+ve and ES-ve).

Intermediates

Intermediate 1: 6α, 9α-Difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1, 4-diene-17β-carbothioic acid diisopropylethylamine salt

A stirred suspension of 6α, 9α-difluoro-11β, 17α-dihydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid (prepared in accordance with the procedure described in GB 2088877B) (49.5g) in methylacetate (500ml) is treated with triethylamine (35ml) maintaining a reaction temperature in the range 0-5°C. 2-Furoyl chloride (25ml) is added and the mixture stirred at 0-5°C for 1 hour. A solution of diethanolamine (52.8g) in methanol (50ml) is added and the mixture stirred at 0-5°C for at least 2 hours. Dilute hydrochloric acid (approx 1M, 550ml) is added maintaining a reaction temperature below 15°C and the mixture stirred at 15°C. The organic

phase is separated and the aqueous phase is back extracted with methyl acetate (2x250ml). All of the organic phases are combined, washed sequentially with brine (5 x 250ml) and treated with di-isopropylethylamine (30ml). The reaction mixture is concentrated by distillation at atmospheric pressure to an approximate volume of 250ml and cooled to 25-30°C (crystallisation of the desired product normally occurs during distillation/subsequent cooling). Tertiary butyl methyl ether (TBME) (500ml) is added, the slurry further cooled and aged at 0-5°C for at least 10 minutes. The product is filtered off, washed with chilled TBME (2x200ml) and dried under vacuum at approximately 40-50°C (75.3g, 98.7%). NMR ($CDCl_3$) δ: 7.54-7.46 (1H, m), 7.20-7.12 (1H, dd), 7.07-6.99 (1H, dd), 6.48-6.41 (2H, m), 6.41-6.32 (1H, dd), 5.51-5.28 (1H, dddd $^2J_{H-F}$ 50Hz), 4.45-4.33(1H, bd), 3.92-3.73 (3H, bm), 3.27-3.14 (2H, q), 2.64-2.12 (5H, m), 1.88-1.71 (2H, m), 1.58-1.15 (3H, s), 1.50-1.38 (15H, m), 1.32-1.23 (1H, m), 1.23-1.15 (3H s), 1.09-0.99 (3H, d)

Intermediate 2: 6α, 9α-Difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester Unsolvated Form 1

A mobile suspension of Intermediate 1 (12.61g, 19.8mmol) in ethyl acetate (230ml) and water (50ml) is treated with a phase transfer catalyst (benzyltributylammonium chloride, 10mol%), cooled to 3°C and treated with bromofluoromethane (1.10ml, 19.5mmol, 0.98 equivalents), washing in with prechilled (0°C) ethyl acetate (EtOAc) (20ml). The suspension is stirred overnight, allowing to warm to 17°C. The aqueous layer is separated and the organic phase is sequentially washed with 1M HCl (50ml), 1%w/v $NaHCO_3$ solution (3x50ml) and water (2x50ml). The ethylacetate solution is distilled at atmospheric pressure until the distillate reaches a temperature of approximately 73°C at which point toluene (150ml) is added. Distillation is continued at atmospheric pressure until all remaining EtOAc has been removed (approximate distillate temperature 103°C). The resultant suspension is cooled and aged at <10°C and filtered off. The bed is washed with toluene (2x30ml) and the product oven dried under vacuum at 60°C to constant weight to yield the title compound (8.77g, 82%) LCMS retention time 3.66min, *m/z* 539 MH^+, NMR δ ($CDCl_3$) includes 7.60 (1H, m), 7.18 – 7.11 (2H, m), 6.52 (1H, dd, *J* 4.2Hz), 6.46 (1H, s), 6.41 (1H, dd, *J* 10, 2Hz),

5.95 and 5.82 (2H dd, *J* 51, 9Hz), 5.48 and 5.35 (1H, 2m), 4.48 (1H, m), 3.48 (1H, m), 1.55 (3H, s), 1.16 (3H, s), 1.06 (3H, d, *J* 7Hz).

Pharmacological Activity

In Vitro Pharmacological Activity

Pharmacological activity was assessed in a functional *in vitro* assay of glucocorticoid agonist activity which is generally predictive of anti-inflammatory or anti-allergic activity *in vivo*.

For the experiments in this section, compound of formula (I) was used as unsolvated Form 1 (Intermediate 2) .

The functional assay was based on that described by K.P.Ray et al., Biochem J. (1997), **328**, 707-715. A549 cells stably transfected with a reporter gene containing the NF-κB responsive elements from the ELAM gene promoter coupled to sPAP (secreted alkaline phosphatase) were treated with test compounds at appropriate doses for 1 hour at 37°C. The cells were then stimulated with tumour necrosis factor (TNF, 10ng/ml) for 16 hours, at which time the amount of alkaline phosphatase produced is measured by a standard colourimetric assay. Dose response curves were constructed from which EC_{50} values were estimated.

In this test the compound of formula (I) showed an EC_{50} value of <1nM.

The glucocorticoid receptor (GR) can function in at least two distinct mechanisms, by upregulating gene expression through the direct binding of GR to specific sequences in gene promotors, and by downregulating gene expression that is being driven by other transcription factors (such as NFκB or AP-1) through their direct interaction with GR.

In a variant of the above method, to monitor these functions, two reporter plasmids have been generated and introduced separately into A549 human lung epithelial cells by transfection. The first cell line contains the firefly luciferase reporter gene under the control of a synthetic promoter that specifically responds to activation of the transcription factor NFκB when stimulated with TNFα. The second cell line contains the renilla luciferase reporter gene under the control of a synthetic promotor that

comprises 3 copies of the consensus glucocorticoid response element, and which responds to direct stimulation by glucocorticoids. Simultaneous measurement of transactivation and transrepression was conducted by mixing the two cell lines in a 1:1 ratio in 96 well plate (40,000 cells per well) and growing overnight at 37°C. Test compounds were dissolved in DMSO, and added to the cells at a final DMSO concentration of 0.7%. After incubation for 1h 0.5ng/ml TNFα (R&D Systems) was added and after a further 15 hours at 37°C, the levels of firefly and renilla luciferase were measured using the Packard Firelite kit following the manufacturers' directions. Dose response curves were constructed from which EC_{50} values were determined.

	Transactivation (GR)	Transrepression (NFκB)
	ED_{50} (nM)	ED_{50} (nM)
Compound of Formula (I)	0.06	0.20
Metabolite (X)	>250	>1000
Fluticasone propionate	0.07	0.16

In Vivo Pharmacological Activity

Pharmacological activity *in vivo* was assessed in an ovalbumin sensitised Brown Norway rat eosinophilia model. This model is designed to mimic allergen induced lung eosinophilia, a major component of lung inflammation in asthma.

For the experiments in this section, compound of formula (I) was used as unsolvated Form 1.

Compound of formula (I) produced dose dependant inhibition of lung eosinophilia in this model after dosing as an intra-tracheal (IT) suspension in saline 30 min prior to ovalbumin challenge. Significant inhibition is achieved after a single dose of 30µg of compound of formula (I) and the response was significantly ($p=0.016$) greater than that seen with an equivalent dose of fluticasone propionate in the same study (69% inhibition with compound of formula (I) *vs* 41% inhibition with fluticasone propionate).

In a rat model of thymus involution 3 daily IT doses of 100μg of compound (I) induced significantly smaller reductions in thymus weight (p= 0.004) than an equivalent dose of fluticasone propionate in the same study (67% reduction of thymus weight with compound (I) *vs* 78% reduction with fluticasone propionate).

Taken together these results indicate a superior therapeutic index for compound (I) compared to fluticasone propionate.

In vitro metabolism in rat and human hepatocytes

Incubation of compound (I) with rat or human hepatocytes shows the compound to be metabolised in an identical manner to fluticasone propionate with the 17-β carboxylic acid (X) being the only significant metabolite produced. Investigation of the rate of appearance of this metabolite on incubation of compound (I) with human hepatocytes (37°C, 10μM drug concentration, hepatocytes from 3 subjects, 0.2 and 0.7 million cells/mL) shows compound (I) to be metabolised *ca.* 5-fold more rapidly than fluticasone propionate:-

Subject number	Cell density (million cells/mL)	17-β acid metabolite production (pmol/h)	
		Compound (I)	Fluticasone propionate
1	0.2	48.9	18.8
1	0.7	73.3	35.4
2	0.2	118	9.7
2	0.7	903	23.7
3	0.2	102	6.6
3	0.7	580	23.9

Median metabolite production 102-118 pmol/h for compound (I) and 18.8-23.0 pmol/h for fluticasone propionate.

Pharmacokinetics after intravenous (IV) and oral dosing in rats

Compound (I) was dosed orally (0.1mg/kg) and IV (0.1 mg/kg) to male Wistar Han rats and pharmacokinetic parameters determined. Compound (I) showed negligible oral bioavailability (0.9%) and plasma clearance of 47.3 mL/min/kg, approaching liver blood flow (plasma clearance of fluticasone propionate = 45.2 mL/min/kg).

Pharmacokinetics after intra-tracheal dry powder dosing in the pig.

Anaesthetised pigs (2) were dosed intra-tracheally with a homogenous mixture of compound (I) (1mg) and fluticasone propionate (1mg) as a dry powder blend in lactose (10% w/w). Serial blood samples were taken for up to 8h following dosing. Plasma levels of compound (I) and fluticasone propionate were determined following extraction and analysis using LC-MS/MS methodology, the lower limits of quantitation of the methods were 10 and 20pg/mL for compound (I) and fluticasone propionate respectively. Using these methods compound (I) was quantifiable up to 2 hours after dosing and fluticasone propionate was quantifiable up to 8 hours after dosing. Maximum plasma concentrations were observed for both compounds within 15min after dosing. Plasma half-life data obtained from IV dosing (0.1mg/kg) was used to calculate AUC (0-inf) values for compound (I). This compensates for the plasma profile of Compound (I) only being defined up to 2 hours after an IT dose and removes any bias due to limited data between compound (I) and fluticasone propionate.

C_{max} and AUC (0-inf) values show markedly reduced systemic exposure to compound (I) compared to fluticasone propionate:-

	Cmax (pg/mL)		AUC (0-inf) (hr.pg/mL)	
	Pig 1	Pig 2	Pig 1	Pig 2
Compound of Formula (I)	117	81	254	221
Fluticasone propionate	277	218	455	495

The pharmacokinetic parameters for both compound (I) and fluticasone propionate were the same in the anaesthetised pig following intravenous administration of a

mixture of the two compounds at 0.1mg/kg. The clearance of these two glucocorticoids is similar is this experimental pig model.

Examples

Example 1 : Nasal formulation containing 6α, 9α-Difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester

A formulation for intranasal delivery was prepared with ingredients as follows:

6α, 9α-Difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester (prepared according to Intermediate 2, micronised to MMD 3μm)	0.05% w/w
Polysorbate 80	0.025% w/w
Avicel RC591	1.5% w/w
Dextrose	5.0% w/w
BKC	0.015% w/w
EDTA	0.015% w/w
water	to 100%

in a total amount suitable for 120 actuations and the formulation was filled into a bottle (plastic or glass) fitted with a metering valve adapted to dispense 50 or 100 μl per actuation

The device was fitted into a nasal actuator (Valois, e.g. VP3, VP7 or VP7D)

The formulation was prepared following the following protocol:

Part A

1. Dissolve dextrose in purified water
2. Dissolve EDTA in dextrose solution
3. Add Avicel RC591 while stirring
4. Allow suspension to hydrate

Part B (separately)

1. Dissolve polysorbate 80 in purified water at 50-60 °C.
2. Prepare slurry of drug in Polysorbate 80 solution

Part C

1. Combine suspension of A4 with suspension of B2 and stir

2. Add solution of BKC in purifed water and stir
3. Adjust pH with 1N HCl
4. Add purified water to correct weight

Example 2 : Nasal formulation containing 6α, 9α-Difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester

A formulation for intranasal delivery was prepared with ingredients as follows:

6α, 9α-Difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester (prepared according to Intermediate 2, micronised to MMD 3μm)	0.1% w/w
Polysorbate 80	0.025% w/w
Avicel RC591	1.5% w/w
Dextrose	5.0% w/w
BKC	0.015% w/w
EDTA	0.015% w/w
water	to 100%

in a total amount suitable for 120 actuations and the formulation was filled into a bottle fitted with a metering valve adapted to dispense 50 or 100 μl per actuation. The device was fitted into a nasal actuator (Valois).

Stability studies on Examples 1 and 2 showed them to be stable up to 3 months at 40 °C (measurements were not taken beyond this time).

Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer or step or group of integers but not to the exclusion of any other integer or step or group of integers or steps.

The patents and patent applications described in this application are herein incorporated by reference.